Gut microbiome alterations at acute myeloid leukemia diagnosis are associated with muscle weakness and anorexia

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Abstract

The gut microbiota makes critical contributions to host homeostasis, and its role in the treatment of acute myeloid leukemia (AML) has attracted attention. We investigated whether the gut microbiome is affected by AML, and whether such changes are associated with hallmarks of cachexia. Biological samples and clinical data were collected from 30 antibiotic-free AML patients at diagnosis and matched volunteers (1:1) in a multicenter, cross-sectional, prospective study. The composition and functional potential of the fecal microbiota were analyzed using shotgun metagenomics. Fecal, blood, and urinary metabolomics analyses were performed. AML patients displayed muscle weakness, anorexia, signs of altered gut function, and glycemic disorders. The composition of the fecal microbiota differed between patients with AML and control subjects, with an increase in oral bacteria. Alterations in bacterial functions and fecal metabolome support an altered redox status in the gut microbiota, which may contribute to the altered redox status observed in patients with AML. *Eubacterium eligens*, reduced 3-fold in AML patients, was strongly correlated with muscle strength and citrulline, a marker of enterocyte mass and function. *Blautia* and *Parabacteroides*, increased in patients with AML, were correlated with anorexia. Several bacterial taxa and metabolites (e.g., *Blautia*, *Prevotella*, phenylacetate, and hippurate) previously associated with glycemic disorders were altered. Our work revealed important perturbations in the gut microbiome of AML patients at diagnosis, which are associated with muscle strength, altered redox status, and anorexia. These findings pave the way for future mechanistic work to explore the function and therapeutic potential of the bacteria identified in this study.

Introduction

The role of the gut microbiota in acute myeloid leukemia (AML) has attracted the attention of the scientific community. The complex microbiota community inhabiting the human gastrointestinal tract cultivates an intricate and mutually beneficial relationship with its host. The gut microbiota synthesizes essential metabolites and releases bioactive

compounds, which can influence host physiology.[1] Fifteen percent of the metabolites found in mammalian blood are derived from the gut microbiota,[2] underlying the profound imbalance between the host and gut microbiota metabolism. The gut microbiota can also regulate host metabolism and immunity through the regulation of gut function.[3] Therefore, gut microbiota is considered a key regulator of host metabolism and inflammatory status in different pathophysiological contexts, including metabolic syndrome and insulin resistance.[4]

AML is a clonal disorder of hematopoietic stem cells, resulting in impaired production of the myeloid blood cell lineage. The standard treatment for younger AML patients (<65 years) is intensive induction chemotherapy followed by consolidation chemotherapy and, in some cases, allogeneic hematopoietic stem cell transplantation. The gut microbiota seems to control aspects of hematopoiesis through mechanisms including metabolite production as well as circulating microbial DNA.[5] AML is also characterized by an altered host immunity, this latter being widely influenced by the gut microbiota. Despite the expected links between AML and gut microbiota, little is known about the influence of AML on the composition of the gut microbiota, whereas more is known about the impact of chemotherapy.

Indeed, patients undergoing induction chemotherapy experience loss of microbiota diversity.[6,7] The reduction in some beneficial bacterial species, such as *Faecalibacterium prausnitzii* and *Bifidobacterium spp*., was found to persist even after completion of chemotherapy.[8] A higher microbial diversity at diagnosis was linked to a reduced risk of infectious complications following induction chemotherapy, suggesting that microbiota profiling could be useful in infectious risk stratification.[9] Much of the recent work investigating the gut microbiota in the context of AML has focused on its relationship with the development of graft-*versus*-host disease (GvHD) after allogeneic hematopoietic stem cell transplantation. Higher α-diversity was associated with better survival[10] and higher levels of *Blautia* with reduced GvHD-related mortality.[11] The presence of *Eubacterium limosum* is also associated with a reduced risk of disease relapse.[12] During the post-transplant period, the presence of antibiotic-resistant bacteria or domination by some bacteria, such as *Enterococcus spp*. and *Proteobacteria*, is associated with a higher risk of infection.[13,14] These observations have encouraged the evaluation of strategies to enhance microbial diversity. In this context, a recent study investigated the safety and diversity-enhancing ability of autologous fecal microbiota transfer in patients with AML receiving intensive chemotherapy and antibiotics.[15] Fecal material collected at the time of diagnosis was used for fecal microbiota transfer. This transfer appeared to be safe and could restore microbial richness and diversity based on α-diversity indices.

Many unknowns remain concerning the composition and activity of gut microbiota at diagnosis and its potential influence on the host in AML. In a mouse model of leukemia, we showed that gut microbiota composition was altered alongside the gut barrier and that reversing these changes through administration of probiotics improved features of cachexia.[16,17] Cachexia is a multifactorial systemic syndrome characterized by substantial weight loss (coming from atrophy of the skeletal muscle and adipose tissue), often accompanied by anorexia, muscle weakness, insulin resistance, and inflammation, which occurs in approximately 50-80% of patients with cancer.[18] Although cachexia affects 40% of patients with hematologic cancers and is often witnessed in clinics, studies focusing on AML-related cachexia are scarce.[19] Therefore, we launched an academic, multicenter, cross-sectional, prospective study to evaluate gut microbiota composition and activity in AML patients and volunteers matched for age, sex, body mass index (BMI) and smoking status. Thirty patients newly diagnosed with AML were recruited before therapeutic intervention. We collected information on the patients' food habits and hallmarks of cachexia. We also assessed the inflammatory status of the patients, as well as metabolic and gut function markers. Through a multi-omics integrative approach combining metagenomics, fecal, blood, and urinary metabolomics, and clinical data, we investigated the gut microbiota of AML patients at diagnosis and links to hallmarks of cachexia.

Methods

Subjects

Thirty patients newly diagnosed with AML were recruited between December 2015 and December 2019 from three Belgian University hospitals (Saint-Luc Brussels [n=13], UZ Leuven [n=15], and UZ Gent [n=2]). The exclusion criteria included antibiotic consumption within the preceding 30 days, chronic intestinal diseases, obesity (BMI >30), pregnancy, gastric bypass, and treatment with antidiabetic or hypoglycemic drugs.

Control subjects from the general population were recruited between December 2017 and January 2020 based on the same inclusion/exclusion criteria, except for the AML diagnosis. They were matched (1:1) for age, sex, BMI, and smoking status, all factors known to affect the gut microbiota. This study followed the ethical guidelines set out in the Declaration of Helsinki, was approved by the "Comité d'éthique Hospitalo-facultaire des Cliniques Universitaires Saint-Luc" (B403201317128), and all participants provided written informed consent. A full description of the study design is provided in the *Online Supplementary Supporting Information*. The study was retrospectively registered on March 20, 2018 on Clinicaltrials.gov: NCT03881826.

Samples, data collection and analyses

All biological sampling and data collection were performed at diagnosis (i.e., before the initiation of chemotherapy and

antibiotic treatment). The full details are provided in the *Online Supplementary Supporting Information*.
Biochemical, gut microbiota, proton nuclear magnetic resonance (^{1}H-NMR) and statistical analyses are detailed in the *Online Supplementary Supporting Information*. Statistical significance was set at $P<0.05$ and q-value <0.1.

Results

Control subjects and acute myeloid leukemia patients have similar anthropometric characteristics and habits

As ensured by one-to-one matching, the group of controls and the group of AML patients did not show any differences in terms of age, sex, BMI, body composition, and percentage of smokers (Table 1). They also had similar habits in terms of daily alcohol consumption. The food frequency questionnaire[20] revealed no differences in the overall dietary scores. No differences in drug exposure, food supplement consumption, and transit scores were detected between the groups (*Online Supplementary Table S1, Online Supplementary Figure S1*).

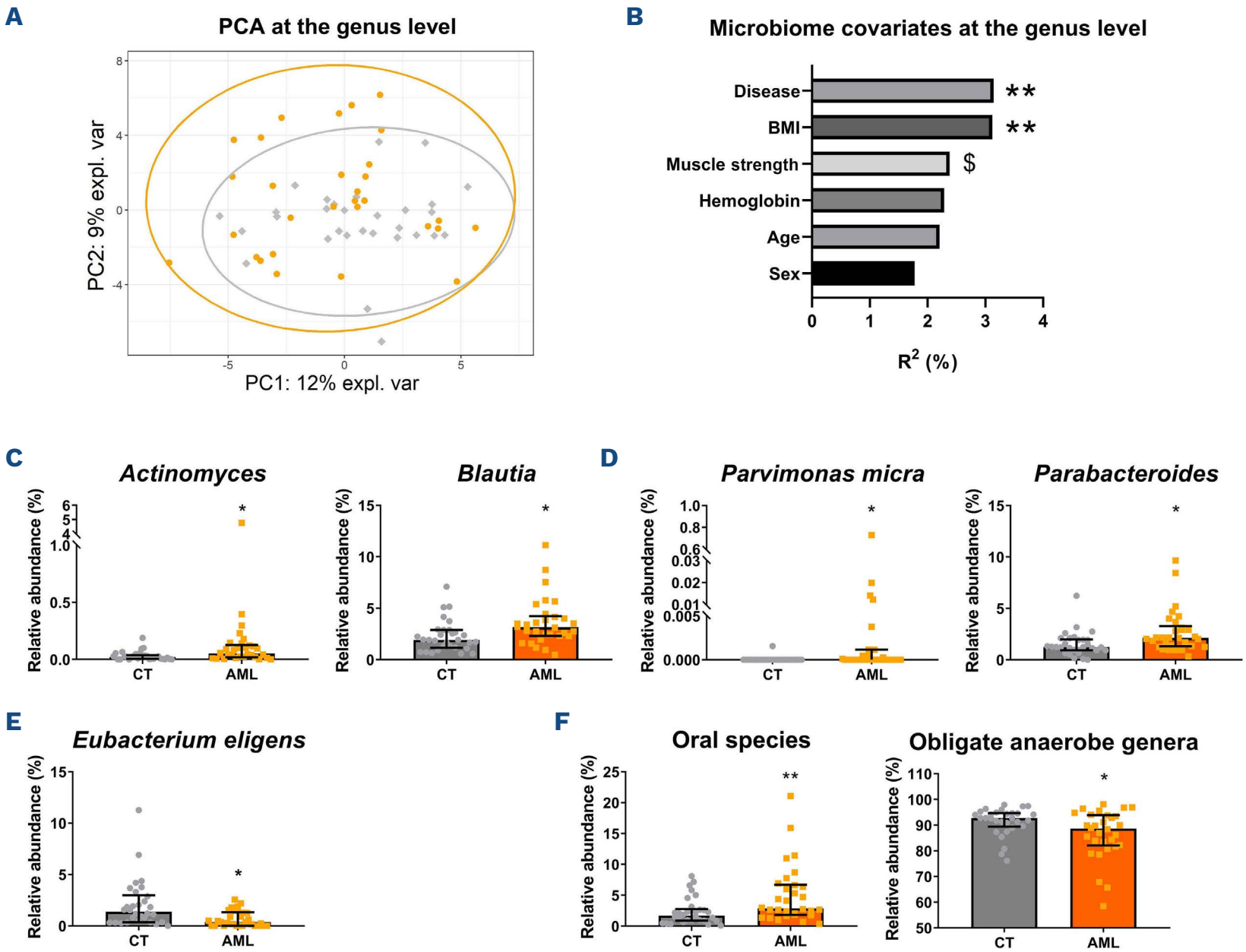
Patients with acute myeloid leukemia have specific gut microbiota alterations

The composition of the gut microbiota was assessed in the same stool samples using two independent techniques (16S rRNA gene sequencing and metagenomics). Gut microbiota composition was not differentially clustered between controls and AML patients, as revealed by principal component analysis at the genus level (Figure 1A, *Online Supplementary Figure S2A*). Permutational multivariate analysis of variance (PERMANOVA) revealed that 3.1% of the variation in gut microbiota composition, as assessed at the genus level by shotgun metagenomics, was explained by AML (Figure 1A, B). At the species level, the disease explained 3.2% of the variation of the dataset (*Online Supplementary Figure S3A*). We also assessed the explanatory power of other factors previously shown to explain most of the variation in gut microbiota composition in the Belgian Flemish Gut Flora Project, a cohort of 1,106 Belgian volunteers,[21] namely BMI, hemoglobin, and age (Figure 1B, *Online Supplementary Figure S3B*). Disease presence had the highest explanatory power, close to BMI (3.1% in this cohort and ~3% in the Belgian Flemish Gut Flora Project) while age and sex were not significant. Total bacterial levels were similar in the two groups (*Online Supplementary Figure S2B*), as were the α-diversity indices (*Online Supplementary Figure S2C*). However, the univariate analyses revealed specific changes. Three genera, namely *Actinomyces, Blautia* and *Parabacteroides*, and one species, *Parvimonas micra*, were increased in the feces of patients with AML, whereas *Eubacterium eligens* was decreased (Figure 1C). These changes, highlighted by shotgun metagenomics, were confirmed by 16S rRNA gene sequencing, with the exception of *Blautia* (*Online Supplementary Figure S2D*, *Online Supplementary Table S2*). *Coprococcus* and *Prevotella* were reduced in AML patients as assessed by 16S rRNA gene sequencing (q=0.109 using shotgun metagenomics) (*Online Supplementary Figure S2D*, *Online Supplementary Table S2*). The

Table 1. Study participants have similar characteristics at baseline.

Characteristics	Total N=60	Controls N=30	AML patients N=30
Age in years,[a] median (IQR)	59.0 (51.0-67.8)	60.5 (50.0-67.3)	59.0 (52.8-68.8)
Sex, % Female Male	 46.7 53.3	 46.7 53.3	 46.7 53.3
BMI, kg/m^2, mean (SD)	25.0 (3.2)	25.0 (3.2)	25.1 (3.2)
Lean mass, %, mean (SD)	70.7 (8.4)	71.6 (8.1)	69.7 (8.7)
Fat mass, %, mean (SD)	25.6 (8.8)	24.6 (8.5)	26.6 (9.1)
Smoker, %	15	13.3	16.7
Alcohol per day,[a] g, median (IQR)	8.6 (0.0-20.0)	8.2 (2.5-18.6)	9.7 (0.0-23.1)
Overall dietary score, mean (SD)	57.3 (11.2)	59.0 (10.4)	55.4 (12.0)
Dietary quality score,* mean (SD)	62.0 (18.6)	68.8 (14.9)	54.5 (19.6)
Dietary diversity score, mean (SD)	43.1 (18.2)	40.7 (16.6)	45.7 (19.8)
Dietary equilibrium score, mean (SD) Adequacy score, mean (SD) Moderation score, mean (SD)	66.9 (11.0) 71.4 (12.2) 92.6 (8.5)	67.6 (10.6) 70.6 (10.7) 94.1 (5.9)	66.2 (11.5) 72.4 (13.8) 91.0 (10.6)

Variables that are normally distributed are expressed as mean (standard deviation) and are tested using a Student *t* test. [a]Variables that are non-normally distributed are expressed as median (interquartile range) and are tested by a Mann-Whitney U-test. Frequency distributions are tested using a Fisher exact test. Daily alcohol intake is missing for one AML patient (N=29). Dietary scores are missing for three AML patients (N=27). *P value <0.01. N: number; AML: acute myeloid leukemia; IQR: interquartile range; BMI : body mass index; SD: standard deviation.



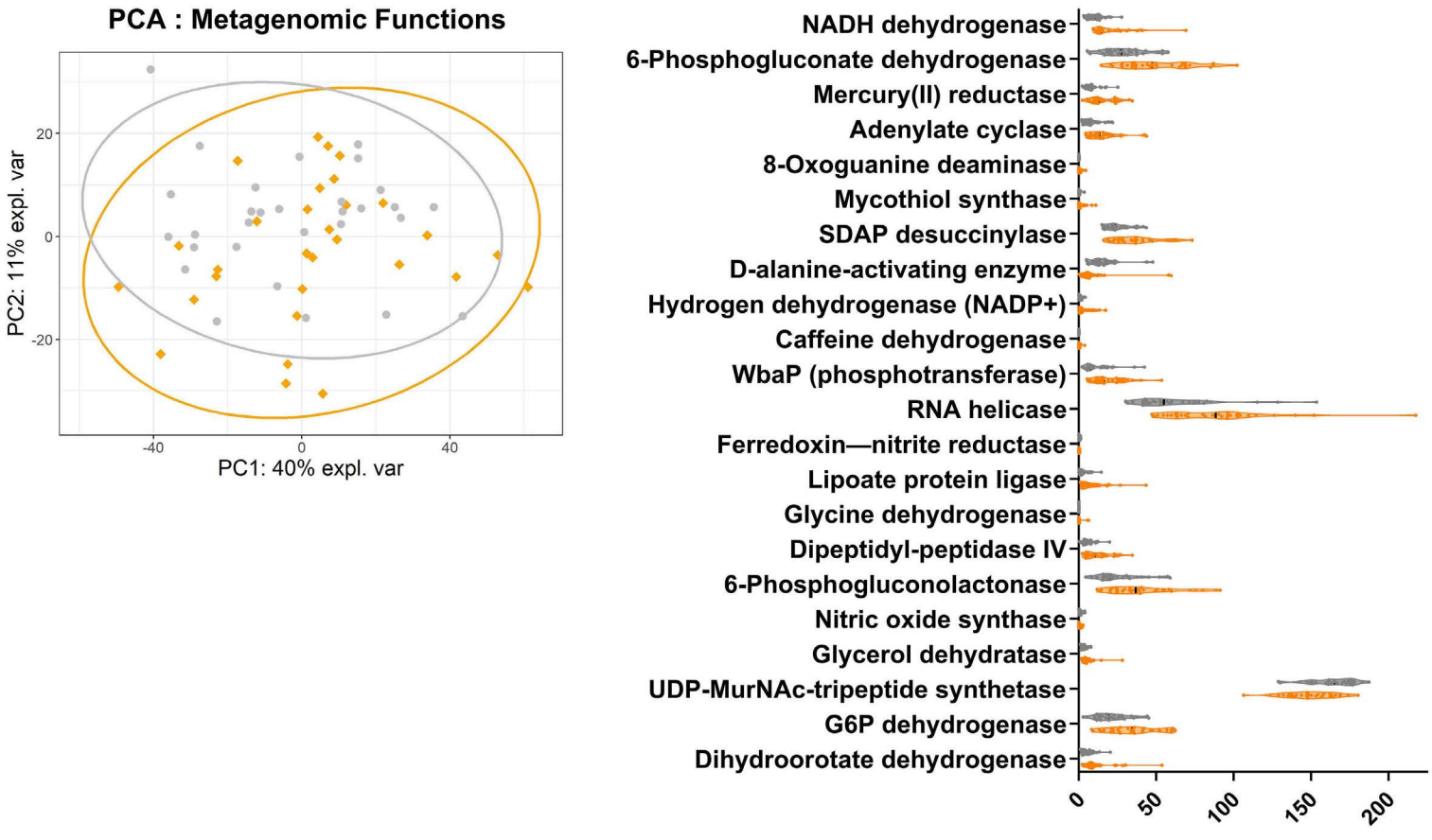


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Figure 1. The composition and function of the gut microbiota of patients with acute myeloid leukemia differ from those of control subjects (results of the metagenomics sequencing). (A) Principal component analysis (PCA) at the genus level (metagenomics results). Permutational multivariate analysis of variance (PERMANOVA): R^2 = 3.1%, *P*<0.01. (B) Contribution of disease, body mass index, muscle strength, hemoglobin, sex, and age to the variance in the PCA at the genus level (PERMANOVA results). $^{**}P$<0.01; $^{\$}P$=0.055. (C) Significantly changed taxa at the lowest taxonomical level from metagenomics results. Mann-Whitney U-tests with a false discovery rate correction were applied. Data are expressed as median with interquartile range. *q value <0.1. (D) Oral species and obligate anaerobe genera. $^{**}P$<0.01; $^{*}P$<0.05. (E) PCA on bacterial EC (Enzyme Commission) enzyme functions. PERMANOVA: R^2=2.1%, not significant. (F) Significantly changed bacterial EC enzyme functions in control subjects (represented in gray) and patients with acute myeloid leukemia (represented in orange). N=30. PCA: principal component analysis; BMI: body mass index; CT: controls; AML: acute myeloid leukemia.

relative abundances of oral species increased more than two-fold, while obligate relative abundances of anaerobe genera were reduced (Figure 1D).

Principal component analysis of metagenomic functions (Figure 1E) confirmed the compositional analyses, with no clear global functional changes between the two groups. However, 22 functions were significantly different (Figure 1F, *Online Supplementary Table S3*). Of these 22 functions, only four were decreased in AML patients, whereas the others were increased. The contributions of the species and genera to each of these functions are shown in *Online Supplementary Figure S4*. A visual inspection of these graphs indicates that for the majority of the functions, the changes in their functional abundances observed in AML patients

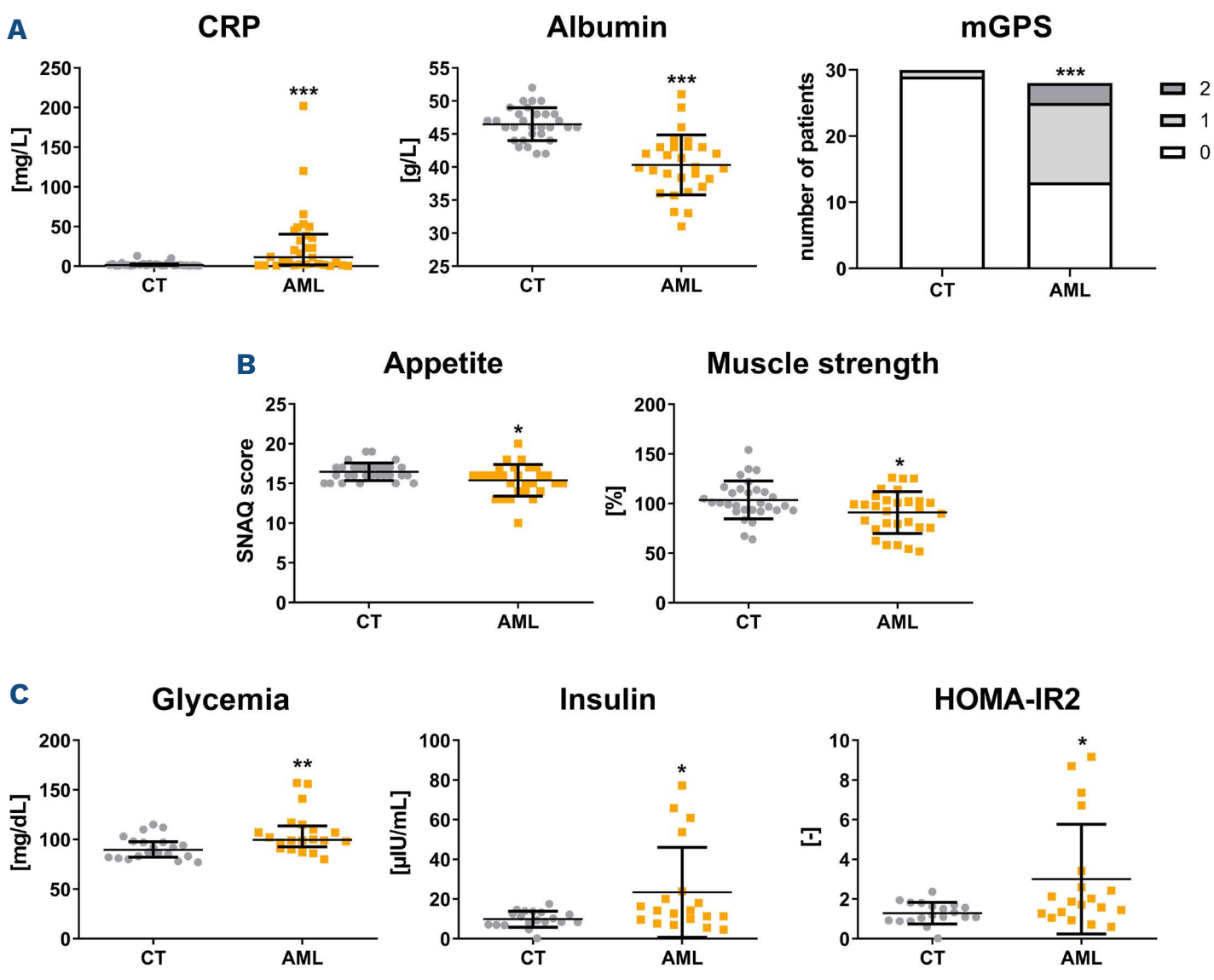

Figure 2. Acute myeloid leukemia patients display anorexia, muscle weakness and glycemic disorders compared to control subjects. (A) C-reactive protein, albumin and modified Glasgow prognostic score in controls and patients with acute myeloid leukemia (AML). (B) Appetite (SNAQ score) and muscle strength in controls and AML. (C) Glycemia (fasted), insulin and HOMA-IR2 in controls and patients with AML. (A, B) N=30. (C) Fasted glycemia, N=20; insulin and HOMA-IR2: N=19. AML patients are represented in orange, controls in gray. Variables that are normally distributed are expressed as mean (standard deviation) and are tested using a Student *t* test or a Welch *t* test. Variables that are non-normally distributed are expressed as median (interquartile range) and are tested by a Mann-Whitney U-test. Differences in modified Glasgow prognostic scores are tested using a χ^2 test. $^{*}P$<0.05; $^{**}P$<0.01; $^{***}P$<0.001. CT: control subjects; AML: acute myeloid leukemia patients; CRP: C-reactive protein; mGPS: modified Glasgow prognostic score; SNAQ: Simplified Nutritional Appetite Questionnaire; HOMA-IR2: second homeostatic model assessment for insulin resistance.

cannot be ascribed to specific species/genera, thereby suggesting that functional changes in the gut microbiota may be independent of compositional changes. These compositional and functional changes were sufficient to predict disease status in a testing set with an accuracy of 87% based on the top altered bacteria and 77% based on the altered functions using random forest models (*Online Supplementary Figure S5*).

Acute myeloid leukemia patients demonstrate anorexia, muscle weakness and glycemic disorders

Patients with AML displayed changes in hemoglobin concentration, white blood cell count, and C-reactive protein, albumin, and glycemia levels characteristic of the disease (Figure 2, *Online Supplementary Figure S6*). The higher C-reactive protein and lower albumin levels also led to a higher modified Glasgow prognostic score (Figure 2A). In addition to

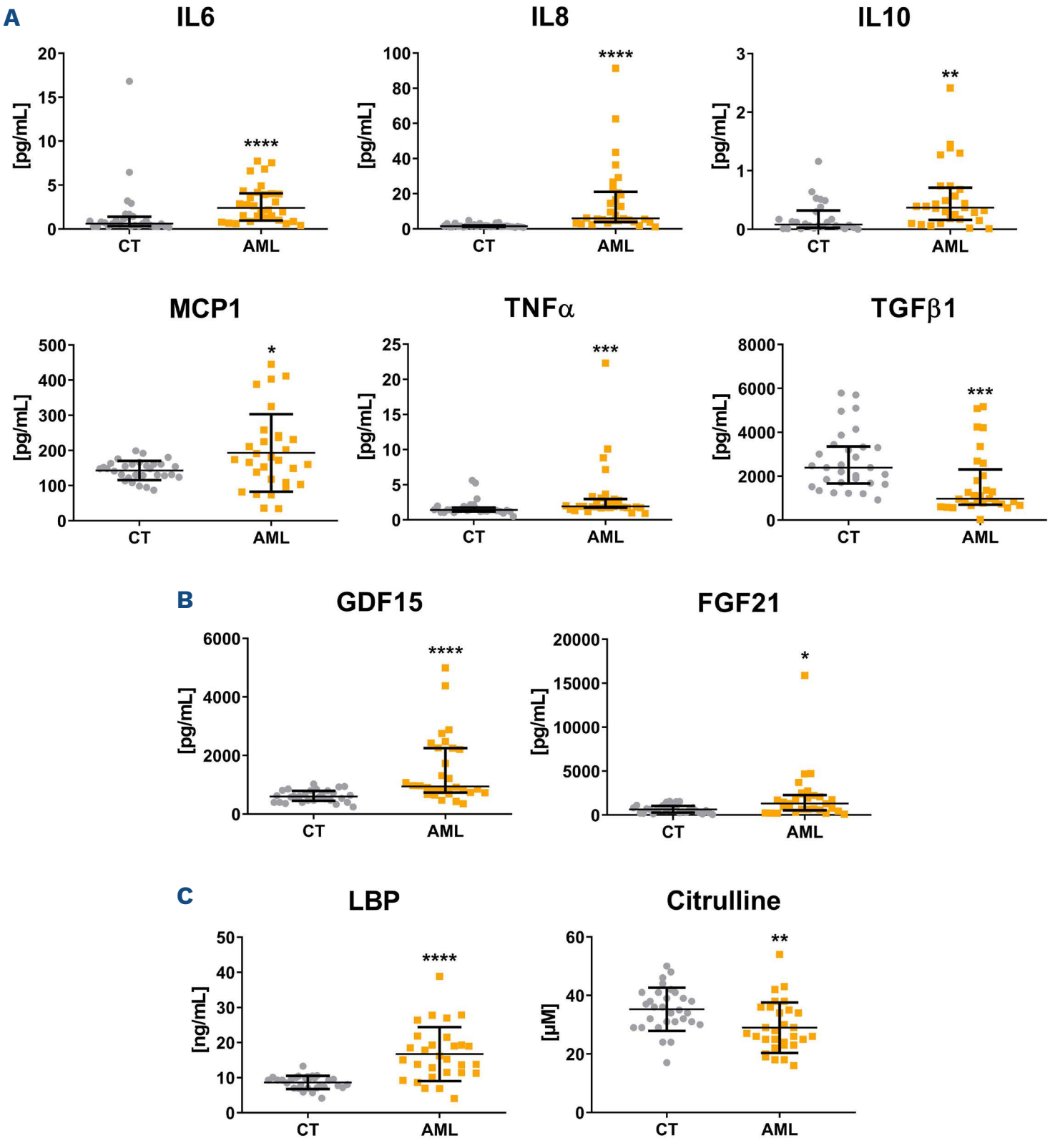

Figure 3. Acute myeloid leukemia patients display inflammation and signs of gut dysfunction compared to control subjects. (A) Inflammatory markers in control subjects and patients with acute myeloid leukemia (AML). (B) Metabolic markers in controls and AML patients. (C) Gut function markers in controls and AML patients. Variables that are normally distributed are expressed as mean (standard deviation) and are tested using a Student *t* test or a Welch *t* test. Variables that are non-normally distributed are expressed as median (interquartile range) and are tested by a Mann-Whitney U-test. AML patients are represented in orange and control subjects in gray. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. CT: control subjects; AML: acute myeloid leukemia patients; IL6: interleukin-6; IL8: interleukin-8; IL10: interleukin-10; MCP1: monocyte chemoattractant protein 1; TNFα: tumor necrosis factor alpha-1; TGFβ1: transforming growth factor beta-1; GDF15: growth differentiation factor 15; FGF21: fibroblast growth factor 21; LBP: lipopolysaccharide-binding protein.

reflecting a higher inflammatory status, the modified Glasgow prognostic score has recently been associated with adverse outcomes in patients with newly diagnosed AML.[22] Appetite score and muscle strength were reduced in patients with AML (Figure 2B). Fasted blood glucose and insulin levels, as well as HOMA-IR2, were higher in AML patients (Figure 2C), collectively reflecting and confirming alterations of glucose metabolism in AML.[23] This appears especially the case for a subset of patients who, intriguingly, presented a specific microbial signature (*Online Supplementary Figures S7* and *S8*; *Online Supplementary Results* and *Discussion*).

In addition to an increase in C-reactive protein, the levels of other inflammatory markers (i.e., interleukin [IL]6), IL8), monocyte chemoattractant protein 1, and tumor necrosis factor α) were elevated in AML patients as compared to the levels in control subjects (Figure 3A). Together with the increase in IL10 and reduction in transforming growth factor β1 (TGFβ1), this confirms alterations in cytokine levels in AML patients[24] and a high inflammatory status. We also reported an increase in growth differentiation factor-15 (GDF15), a member of the TGFβ superfamily, and fibroblast growth factor 21 (FGF21) in AML patients (Figure 3B). GDF15 mediates tumor-induced anorexia and body weight loss[25] while FGF21 has been implicated in fasting-induced muscle atrophy and weakness in mice.[26]

Plasma levels of citrulline, a non-proteinogenic amino acid reflecting enterocyte mass and function,[27] were decreased in patients with AML (Figure 3C). Together with the increased level of lipopolysaccharide-binding protein (Figure 3C), the reduction in citrulline levels in AML patients suggests an alteration of gut function.

Patients with acute myeloid leukemia have specific fecal, blood and urinary metabolome alterations

Principal component analysis performed on fecal, blood, and urinary metabolites did not reveal a clear clustering between controls and AML (*Online Supplementary Figure S9*), with AML samples being more dispersed than the control samples. PERMANOVA revealed that 11.2% and 2.9% of the variation in blood and urine metabolomics, respectively, can be explained by the presence of AML (P<0.05). The metabolites driving the separation between both groups were identified using multivariate discriminant analysis (*Online Supplementary Figure S9*). Univariate analysis also revealed significantly altered metabolites (Figure 4, *Online Supplementary Table S4*). In AML feces, two sugars (glucose and galactose), three alcohols (methanol, ethanol, and glycerol) and two amino acids (phenylalanine and threonine) decreased. Bacterial amino acid-derived metabolites were modified: 3-phenylpropionate decreased, while phenylacetate increased. In AML blood, two sugars (glucose and mannose), as well as ethanol, increased. Amino acids (alanine, asparagine, citrulline, cystine, glutamine, methionine, sarcosine, and threonine) decreased, except for glutamate, which increased. Uridine and urea levels were decreased. Lactate levels increased, while acetate, fumarate, and succinate levels decreased. Amino acid catabolic products, such as 3-hydroxyisovalerate, 2-hydroxybutyrate, formate, and dimethylamine, were increased. Ascorbate, also known as vitamin C, was decreased in AML patients. In the urine of AML patients, the metabolites were mainly increased. This was the case for most amino acids (2-aminobutyrate, 3-aminoisobutyrate, carnitine, isoleucine, leucine, taurine, threonine, and tyrosine) and nucleic base-related metabolites (hypoxanthine and pseudouridine), with the exception of uracil, which decreased. Formate, maleate, and Sumiki's acid also increased, whereas hippurate and trigonelline decreased. Consistent with the alterations in glucose metabolism mentioned earlier, glucose levels were also increased in the AML urine samples.

Altered bacteria and functions in acute myeloid leukemia patients correlate with several fecal, blood and urinary metabolites

Next, we performed correlations between the top altered bacterial taxa/functions and fecal, blood, and urinary metabolite levels. The genus *Eubacterium*, as well as the associated species *E. eligens* and *E. hallii*, showed the strongest correlations with fecal metabolites, especially malonate (negative correlation) and glycerol (positive correlation) (Figure 5). Several blood metabolites were strongly correlated with the different bacterial taxa (Figure 5). Urea was negatively correlated with the *Blautia* genus and positively correlated with the *Sutterellaceae* family and *E. eligens*. Ascorbate levels were negatively correlated with *Clostridium spiroforme*. 3-hydroxyisobutyrate correlated negatively with the *Erysipelatoclostridium* genus, and 2-hydroxybutyrate correlated positively with the *Parabacteroides* genus. The correlations were weaker for urinary metabolites (*Online Supplementary Figure S10*). *E. eligens* displayed the highest number of correlations (all negative), mainly with amino acids. *Parabacteroides* also showed several positive correlations. Most of these correlations were maintained when corrected for age (*Online Supplementary Figure S11*). Among those correlations, fecal malonate and glycerol correlations with *Eubacterium*, *E. eligens*, and *E. hallii* were also found in the healthy control group alone (*Online Supplementary Figure S12*). In the healthy control group, blood urea also correlated with *Blautia* as well as *E. eligens*, and 3-hydroxyisobutyrate correlated with the *Erysipelatoclostridium* genus (*Online Supplementary Figures S13* and *S14*).

Interestingly, when looking at the correlations between altered microbial functions and fecal, blood, and urinary metabolomes, some fecal and blood metabolites demonstrated strong correlations with many different functions (*Online Supplementary Figure S15*). For instance, in feces, glycerol showed strong negative correlations with almost all increased functions in the AML group. In blood, several metabolites showed multiple correlations with bacterial function. Some were positive (e.g., correlation with formate)

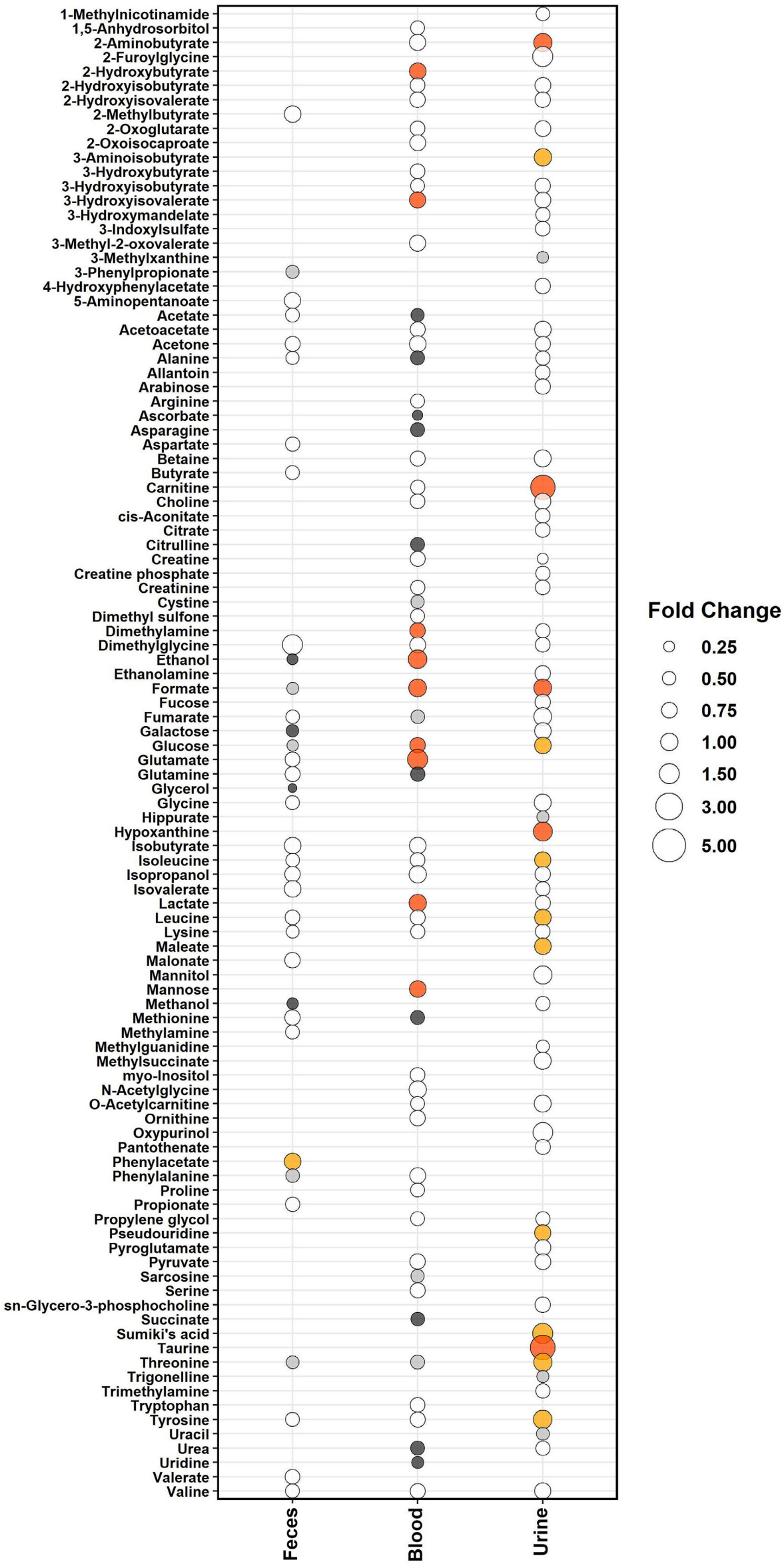

Figure 4. Univariate analyses pinpoint differences in the relative concentrations of identified metabolites in the three analyzed compartments (feces, blood and urine). In the bubble plot. bubble size depicts concentration fold change based on the group median. Colored bubbles correspond to affected metabolites. Light and dark orange are used for metabolites increased in the acute myeloid leukemia (AML) group (with $P<0.05$ and a false discovery rate (FDR)-corrected q-value <0.1). Light and dark gray are used for metabolites decreased in the AML group ($P<0.05$ and FDR-corrected q-value <0.1). Uncolored bubbles represent unaffected metabolites. N=30 per group.

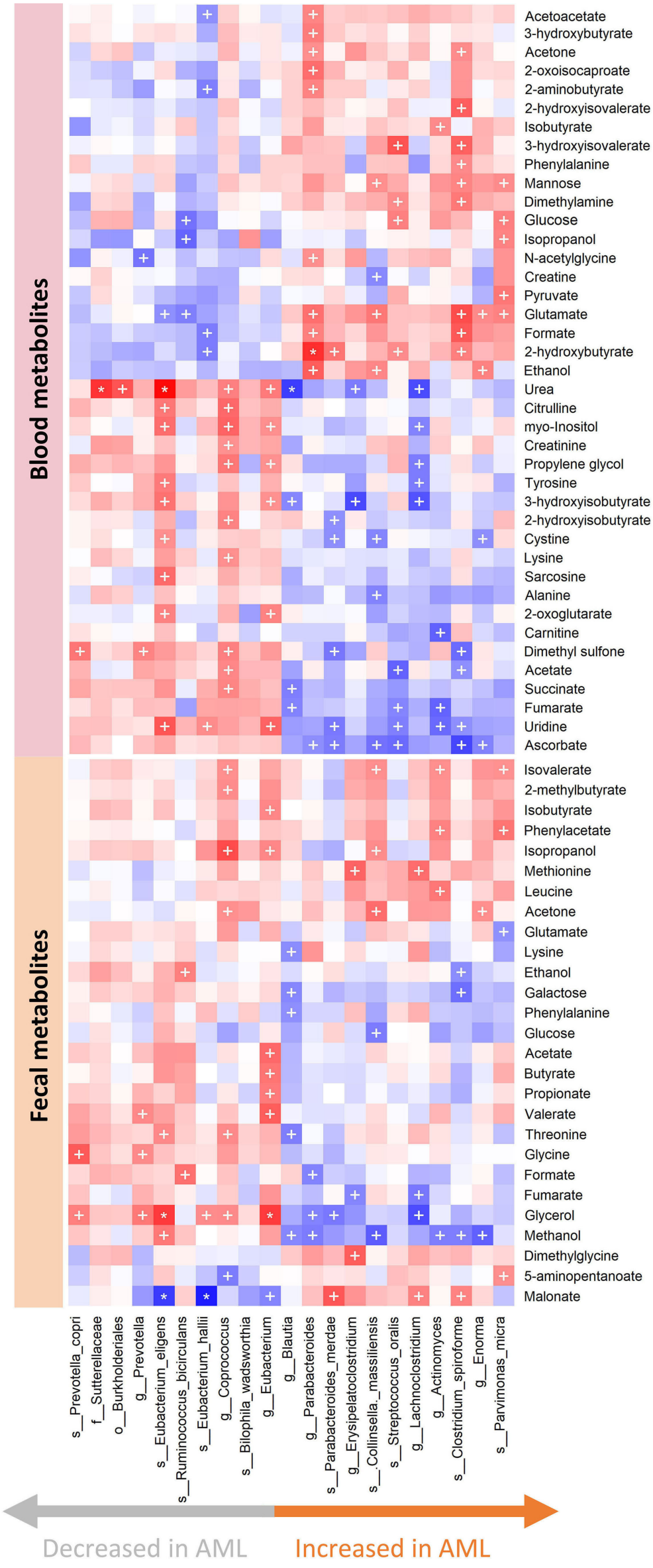

Figure 5. The top altered bacteria correlate with several blood and fecal metabolites. Spearman correlations. Metabolites with at least one correlation with an altered taxon are present. Microbial taxa are ordered by fold change. '+' symbolizes $P<0.05$ and '*' symbolizes a false discovery rate-corrected q-value <0.1. AML: acute myeloid leukemia.

and some were negative (e.g., correlations with ascorbate and propylene glycol). In contrast, ferredoxin-nitrite reductase was negatively correlated with many urinay metabolites, whereas glycerol dehydratase was positively correlated with similar metabolites (*Online Supplementary Figure S16*).

Altered bacteria and functions in acute myeloid leukemia patients associate with clinical, metabolic and inflammatory parameters

We then looked for associations between altered bacterial taxa and functions and the collected clinical, dietary, inflammatory, and metabolic parameters in the whole cohort and exclusively in the AML group to delineate the strongest correlations. We found that many taxa, including *Parvimonas micra*, *Actinomyces*, *Parabacteroides* and *Blautia*, were positively associated with inflammatory markers in the entire cohort (Figure 6). *E. eligens*, decreased in AML patients, was negatively associated with inflammation and positively associated with citrulline and muscle strength; the latter correlation was maintained within the AML group (*Online Supplementary Figure S17*). Consistent with these findings, muscle strength was strongly correlated with blood citrulline (rho=0.3522, *P*=0.0058) and C-reactive protein levels (rho= -0.4585, *P*=0.0002). Looking at the negative correlations, we noticed that *Blautia* was inversely associated with muscle strength. Some parameters, such as hemoglobin, IL6 and IL8 as well as TGFβ1 and citrulline, were associated with many different functions (*Online Supplementary Figure S18*), with some associations maintained when considering exclusively the AML patients (*Online Supplementary Figure S19*). Most of these correlations were maintained when corrected for age (*Online Supplementary Figure S20*).

Discussion

This study highlights alterations in gut microbiota composition and activity, hallmarks of cachexia and metabolic and inflammatory disturbances in patients newly diagnosed with AML, with several associations found between these alterations (Figure 7).

Altered bacterial functions in AML patients include genes catalyzing redox reactions, suggesting an altered redox status in the gut microbiota of such patients. Two elements from fecal metabolomics further support the hypothesis of a redox imbalance in AML gut microbiota towards a more pro-oxidative status. First, phenylalanine, which is decreased in the feces of patients with AML, can be metabolized by the gut microbiota into 3-phenylpropionate and phenylacetate. 3-phenylpropionate, reduced in AML patients, is produced through a reductive pathway, while phenylacetate, which is increased in AML patients, arises through an oxidative pathway.[28] Along these lines, fecal phenylacetate was associated with a higher pro-oxidant and pro-inflammatory status in elderly volunteers[29] whereas blood phenylacetate was elevated in cachectic cancer patients.[30] Second, glycerol levels were reduced in AML feces. This decrease could result from oxidative stress on bacterial membranes, requiring increased lipid synthesis. This is supported by the increase in lipid synthesis functions (glycerol dehydratase and lipoate protein ligase), as well as by the correlations between glycerol and redox and lipid synthesis functions. Notably, the reduction in the relative abundance of obligate anaerobic genera may reflect increased oxygen levels in the intestine, contributing to the altered bacterial redox status. Several elements from blood and urinary metabolomics indicate the presence of oxidative stress on the host side, which is consistent with the findings of previous studies.[31] First, taurine, a non-proteinogenic amino acid with antioxidant properties, was more excreted in the urine of patients with AML. Second, ascorbate, another antioxidant, was decreased in the blood of AML patients compared with its levels in control subjects. Interestingly, a few preclinical studies have demonstrated that the gut microbiota affects the host antioxidant defense system.[32] Considering all these elements, we propose that the altered activity and composition of the gut microbiota observed in AML patients could contribute to the oxidative stress present in these patients. This hypothesis is in line with the results of a previous study arguing for a microbial role in the oxidative stress present in some patients with acute leukemia under chemotherapy. Specifically, the authors proposed oxidative stress as a mediator involved in *Akkermansia*-related neutropenic fever.[33]

In addition to their link with the gut microbiota redox pathways, some metabolites pointed out previously could be linked to altered glycemic homeostasis in AML patients. Indeed, an increase in systemic phenylacetate was causally associated with insulin resistance[34] and 2-hydroxybutyrate, a marker of insulin resistance,[35] was increased in the blood of our cohort of AML patients. Hippurate, a glycine conjugate of microbial benzoate, was decreased in the urine of AML patients. This metabolite is commonly seen as a general marker of metabolic health and is causally associated with glucose tolerance.[36] Hippurate and *Prevotella* increased following the administration of fibre.[37] Their positive correlation in the current study suggests that reduced hippurate levels may be explained by a decrease in *Prevotella*. *Blautia* has been inconsistently associated with different aspects of metabolic syndrome.[38] This genus is increased in anorectic patients.[39] Consistent with this finding, in our study, *Blautia* increased in AML patients and correlated negatively with appetite and positively with the pro-anorexigenic cytokine GDF15. Collectively, our data reinforce previous observations of the contribution of the gut microbiota to metabolic derangements in preclinical models of acute leukemia[40] and suggest that the gut microbiota could influence glucose metabolism and energy intake in AML patients, strengthening the relevance of gut microbiota alterations in this pathological context.

Among all the alterations and correlations with the most

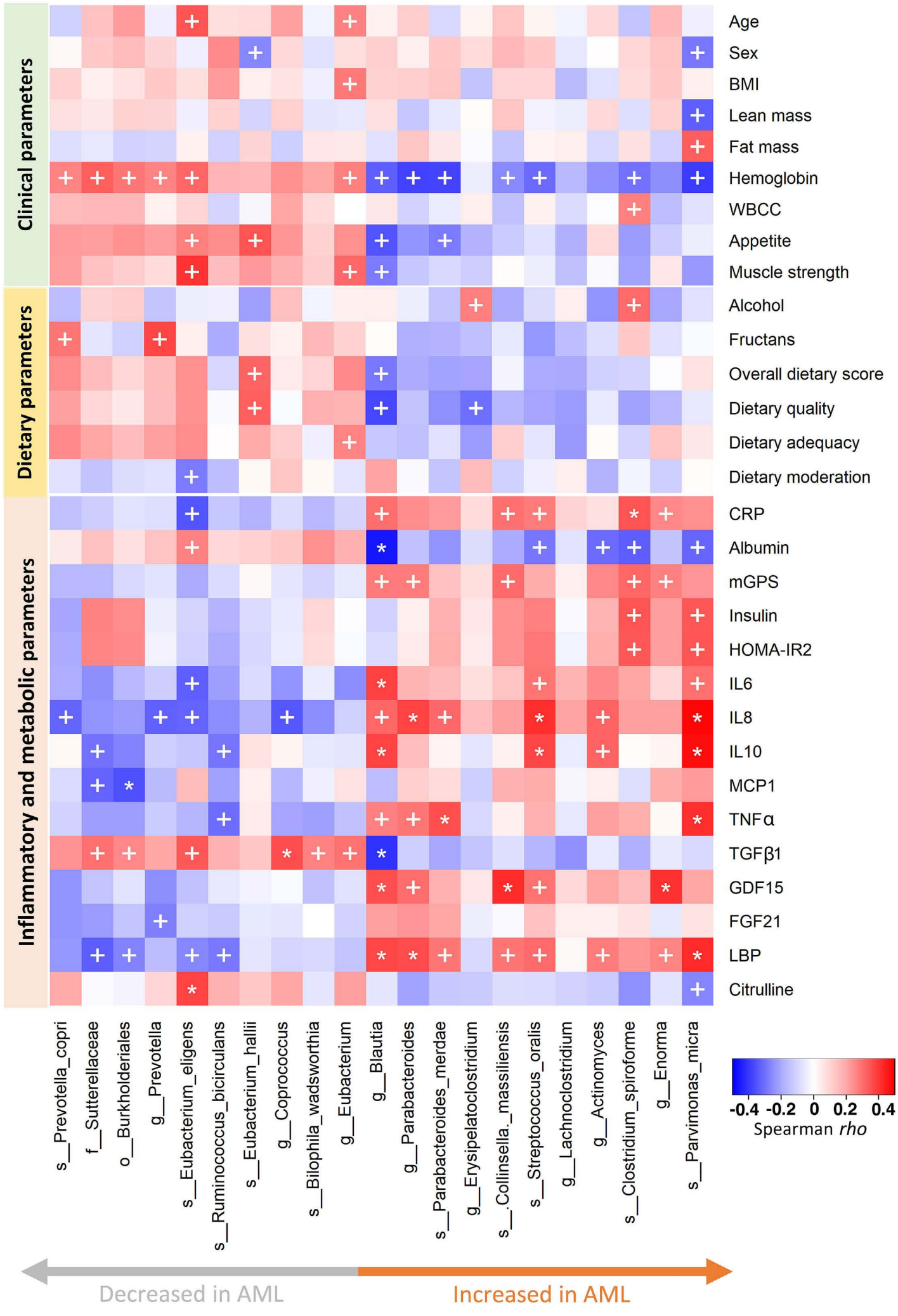

Figure 6. Top altered bacteria correlate with clinical, dietary, inflammatory, and metabolic parameters in control subjects and patients with acute myeloid leukemia. Spearman correlations. '+' symbolizes *P*<0.05 and '*' symbolizes a false discovery rate-corrected q-value <0.1. Parameters with at least one correlation with an altered taxon are present. Microbial taxa are ordered by fold change. BMI: body mass index; WBCC: white blood cell count; appetite (SNAQ score); CRP: C-reactive protein; mGPS: modified Glasgow prognostic score; HOMA-IR2: second homeostatic model assessment for insulin resistance; IL6: interleukin-6; IL8: interleukin-8; IL10: interleukin-10; MCP1: monocyte chemoattractant protein 1; TNFα: tumor necrosis factor alpha; TGFβ1: transforming growth factor beta-1; GDF15: growth differentiation factor 15; FGF21: fibroblast growth factor 21; LBP: lipopolysaccharide-binding protein; AML: acute myeloid leukemia.

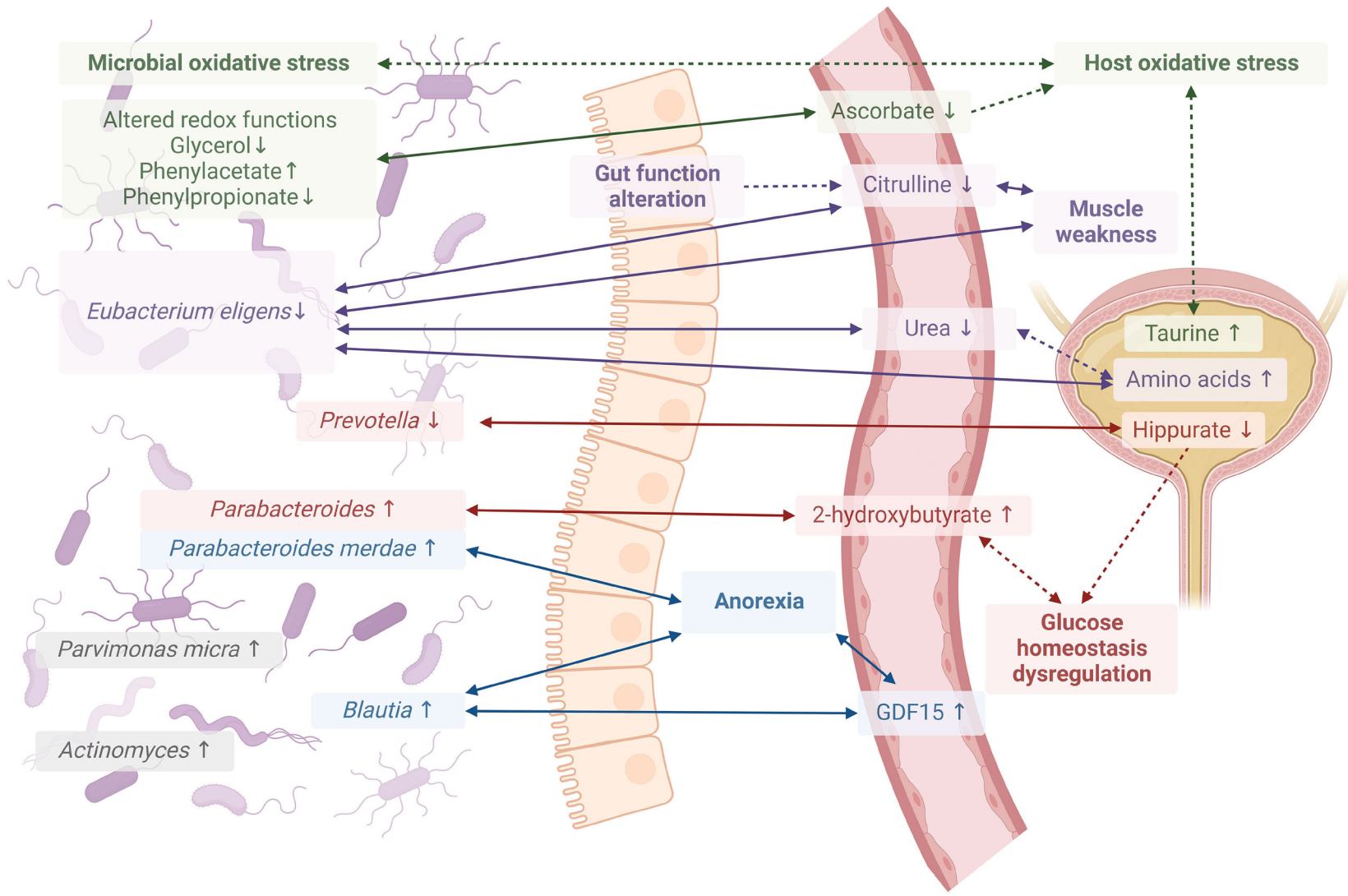

Figure 7. Summary of altered bacteria and functions in acute myeloid leukemia patients and their associations with hallmarks of cachexia and altered host redox status. Full two-way arrows represent significant correlations, dashed two-way arrows signify associations and simple way arrows indicate contributions based on literature. Created with BioRender.com. GDF15: growth differentiation factor 15.

altered bacteria, *E. eligens* stands out. This species, reduced three-fold in leukemic patients compared to controls, was strongly correlated with muscle strength in the entire cohort and when considering exclusively AML patients. Based on urinary metabolomics, we found that *E. eligens* was widely associated with the urinary excretion of amino acids and amino acid metabolites. Higher urinary excretion of carnitine and 2-aminobutyrate was previously associated with cachexia and muscle loss.[41] Blood metabolomics revealed a strong association between *E. eligens* and urea. Such an association with urea, a metabolite involved in the regulation of ammonium levels, may be related to the urinary excretion of amino acids, representing a loss of ammonium. The increase in 8-oxoguanine deaminase and caffeine dehydrogenase, which are two functions strongly correlated with urea, could also indicate greater ammonium use by the gut microbiota. In elderly individuals, *E. eligens* was positively associated with markers of lower frailty,[42] reinforcing the relevance of the association of this bacterial species with muscle strength. Considering the growing awareness of the importance of muscle wasting in AML outcomes,[19] gut microbiota modulation could be an innovative strategy to help patients fight muscle weakness.

E. eligens was also correlated with blood levels of citrulline. Reduction in citrulline levels following intensive chemotherapy was previously reported in AML mice and patients[43] and low plasma citrulline levels before allogeneic hematopoietic stem cell transplantation were associated with a higher risk of gastrointestinal GvHD and non-relapse mortality in patients.[44] A decrease in citrulline combined with an increase in systemic lipopolysaccharide-binding protein levels suggests an alteration of the gut function in AML patients at diagnosis. Gut permeability was not assessed at the functional level in this study as the procedure for ingestible probes is incompatible with the clinical management of patients at diagnosis. An alteration in intestinal functions, such as absorption and gut barrier, could potentially contribute to the inflammation, metabolic disorders and oxidative stress (this last through reduced ascorbate absorption) found in these patients.

Parabacteroides, especially *P. merdae* and *P. distasonis*, were increased in patients with leukemia. These two genera were reported to be affected in many pathologies[38] and were ascribed both beneficial and harmful effects. An increase in *Parabacteroides* was also found in leukemic mice and was attributed to a reduction in food intake.[17] Consistently, in our dataset, *P. merdae* correlated negatively with appetite. In mice with acute leukemia, we found a bloom in members of

the *Enterobacteriaceae* family.[17] In our AML cohort, *Enterobacteriaceae* levels did not change (*Online Supplementary Figure S21*). *Prevotella* was significantly less abundant in leukemic patients, which was mainly explained by a six-fold reduction in *P. copri*, a genus also reduced in cachectic patients.[45] *Actinomyces* and *P. micra* were increased in our cohort of AML patients. These two oral bacteria were previously found to be increased in colorectal cancer patients.[38,46] In line with these studies, the relative abundance of oral species increased more than two-fold. Translocation of oral bacteria to the gut has been associated with many severe diseases, such as inflammatory bowel disease and liver cirrhosis and can promote intestinal inflammation.[47] Reciprocally, we hypothesize that modifications of the microbiota composition observed upon AML are triggered by inflammation, a key driver of microbial dysbiosis.[48,49] This hypothesis is coherent with the positive correlations found between inflammatory cytokines and bacteria increased in AML. Based on preclinical studies,[50] we further speculate that this dysbiosis contributes to disease progression, hence the notion that AML and dysbiosis may be co-evolving.

Our clinical data are consistent with our previous preclinical observations.[16,17] In both settings, we observed metabolic and inflammatory alterations, impaired gut function, and altered gut microbiota composition and function. We previously showed that counteracting microbial alterations using rationally selected prebiotics and/or probiotics, including fibers, improved gut function, and counteracted muscle atrophy in leukemic mice.[16,17] These observations further support the therapeutic potential of gut microbiota-based adjuvant treatments.

This study has several limitations. The sample size was limited, and the data from this exploratory pilot study will need to be confirmed in a larger cohort. As the primary objective of this study was to evaluate gut microbiota composition and function, we enforced several exclusion criteria for metabolic and pathological situations already known to be associated with changes in gut microbiota. The exclusion of patients treated with antibiotics within 30 days and obese patients led us to exclude a considerable proportion of the patients referred to the hospital centers involved in the study. Impedancemetry is less precise than computed tomography scanning for evaluating body composition, but computed tomography scanning was not compatible with the clinical management of patients at diagnosis. In addition, the analysis of skeletal muscle function would have benefited from tests of whole-body strength and range of movement.

In conclusion, our work reveals important alterations in the gut microbiota composition and function of AML patients at diagnosis before any therapeutic intervention. This finding may be of clinical importance considering that autologous fecal material transfer is emerging as an option for these patients.[15] Our findings call for caution when using autologous fecal material transfer during the therapeutic care of AML patients and go in favor of heterologous transfer to increase the gut microbiota diversity and richness in these patients. Whether transfer of fecal material would also be of benefit in cases of cachexia will need to be determined. Gut microbiota alterations are associated with hallmarks of cachexia (muscle weakness, anorexia, and inflammation), redox status, and signs of gut dysfunction. However, association does not imply causality. Therefore, our findings will constitute the basis of future mechanistic studies exploring the contribution and therapeutic potential of the bacteria identified in this study, such as *Eubacterium eligens*.

Disclosures

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Contributions

NMD and LBB conceived and designed the study. VH, J-BD, and HS contributed to the design of the clinical study. SAP, VH, FB, IM, TK, JM, HS, and LBB collected clinical data and biological samples. LBB supervised the work. SAP analyzed the clinical data. SAP performed the metabolomics analysis, with the help of SL for urinary metabolomics. SAP, FL, JW, and LBB performed the microbiome analyses. SAP, ANM, and LBB analyzed metabolic and inflammatory markers. NN conducted the citrulline analysis by mass spectrometry. SAP and LBB integrated and interpreted data and drafted the article. VH, HS, and NMD contributed to data interpretation. LBB acquired funding for the research. All authors revised the article and approved the version to be published.

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Data-sharing statement

The full details of the methods described in this paper are provided in the Online Supporting Information. Raw sequences from 16S rRNA gene sequencing and metagenomics can be found in the SRA database (project ID: PRJNA813705).

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